

Development of a High-Sensitivity Radiation Detector for Chromatography

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Abstract— Radionuclide techniques will be increasingly important for the development of biofuels, since they can both uniquely characterize metabolic pathways and image large model systems. We are investigating the application of nuclear medical imaging tools and techniques to biofuel development, using high-sensitivity chromatographic radiation detectors and compounds radiolabeled with short-lived, cyclotron-produced, positron-emitting isotopes (e.g., ^{11}C). The application of high-sensitivity radiation detectors to standard chromatographic techniques should allow for the measurement of numerous pathway constituents whose levels normally fall below detection limits of conventional instrumentation. In this paper, we describe a proposed parallel-plane PET camera designed for HPLC (high performance liquid chromatography) with over 40 times greater sensitivity than a conventional HPLC radiation detector; this PET camera could be used to image radioactivity in the HPLC exit tube or column. We also present results using a high-sensitivity HPLC radiation detector comprised of 8 HR+ PET detector modules arranged into a parallel plane and read out with HRRT electronics. This high-sensitivity radiation detector was placed in line after a conventional HPLC radiation detector (a small CsI:Ti scintillator crystal coupled to a PIN photodiode). If we inject 9.3 μCi of [^{18}F]FDG into the HPLC system, we see similarly shaped peaks with an excellent signal-to-noise ratio from both radiation detectors. If we inject only 4.1 nCi of [^{18}F]FDG, we measure a signal-to-noise ratio of 27:1 with the high-sensitivity radiation detector and about 3:1 for the conventional radiation detector. We have therefore demonstrated that a high-sensitivity radiation detector, using a parallel-plane PET camera, could become an important tool for fundamental biofuel research.

I. INTRODUCTION

The development of new biofuels requires optimizing the physiological mechanisms involved to enhance the yield and energy value. A key component of this biofuel research is the dynamic measurement of carbon flux down the biofuel-related metabolic pathways (to determine key rate-limiting steps), which can be measured using radiolabeled analogs of pathway substrates and intermediates. Such experiments are usually carried out with long-lived ^{14}C and ^3H isotopes, but their long half-lives limit specific activity and their relatively low decay energies prevent accurate direct measurements. These limitations can be overcome by using short-lived, cyclotron-produced, positron-emitting isotopes with much higher

theoretical specific activities – ^{11}C instead of ^{14}C , and in some cases ^{18}F instead of ^3H . This allows for real-time measurements of carbon flux through pathways, detection of low abundant intermediates, and repetitive experiments on the same plants and cultures. Therefore, we seek to apply nuclear medical imaging tools and techniques to biofuel development. The application of high-sensitivity radiation detectors to standard chromatographic techniques should allow for the measurement of numerous pathway constituents whose levels normally fall below detection limits of conventional instrumentation. This paper focuses on our development of a high-sensitivity HPLC (high performance liquid chromatography) radiation detector for positron-emitting compounds. However, this detector could also be used for radiotracer experiments analyzed by gas chromatography.

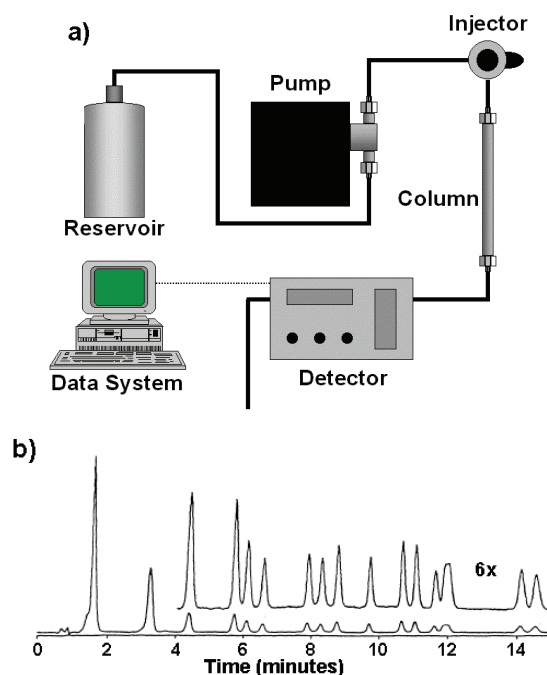


Figure. 1. (a) A typical HPLC system [1]. (b) Typical HPLC data [2], showing concentration (y-axis) as a function of time (x-axis). Each peak corresponds to an individual compound.

A typical HPLC system is shown in Fig. 1a [1]. The initial multi-component solution contains the compounds of interest (e.g., substrates for plant biosynthesis and the resulting biosynthetic products) dissolved in an appropriate solvent. This solution is pumped through a separation column, with the properties of the column chosen so that the transport velocity through the column is different for the different compounds.

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For instance, compounds that are more attracted to the mobile solvent move at a higher velocity (*i.e.*, closer to that of the solvent), whereas compounds more attracted to the stationary particles trapped in the column move significantly slower. Thus, the individual compounds exit the column at different times. Small-diameter tubing carries the compound (and solvent) from the exit of the column into a detector that uses some attribute of the compound, such as its optical absorbance or radioactive concentration, to measure the relative concentration of the compound as a function of time. Thus, the output of the HPLC detector will be similar to Fig. 1b [2]. Assuming that the column has separated the individual compounds, each compound is seen as a single peak in the detector output. The separation between peaks is determined by the properties of the column, solvent, compound, and pumping speed, but typically ranges from a few seconds to minutes. The width of each peak depends on these same properties and varies widely, but it can be as small as several seconds. The ratio of the peak width to separation determines the ability to resolve the individual peaks (*i.e.*, the compounds). The area under each peak is proportional to the concentration of the compound. Naturally, a sensitive detector with good signal-to-noise ratio is desired to accurately measure small concentrations of compounds.

In practice, radiolabeled compounds and a radiation detector are common tools for performing HPLC. The radiation detector is typically a 1 cm diameter CsI:Tl scintillator crystal coupled to a PIN photodiode (Fig. 2). The detector is placed in close proximity to the tubing, and lead shielding is placed outside of the detector/tubing. Although the volume of tubing observed by the radiation detector is small (typically 0.75 mm diameter and ~6 cm long), the sensitivity of the detector is sufficient for many applications. For applications where higher sensitivity is desired, the tubing is coiled around the detector. This increases the length of time that the peak spends near the detector by a factor of n , where n is the number of turns of tubing, and so increases the sensitivity by a factor of n . However, increasing the length of time that the peak spends near the detector also increases the measured width of the peak and so degrades the ability to separate peaks.

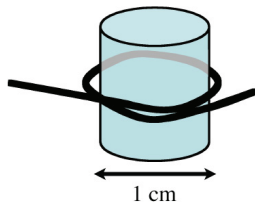


Figure 2. Diagram of a conventional radiation detector used with HPLC. The cylinder represents a CsI:Tl scintillator coupled to a photodiode. One (or more) coils of tubing are wrapped around the scintillator, with both the sensitivity and time resolution (ability to resolve individual peaks) being proportional to the number of coils. The assembly is surrounded by lead shielding (not shown).

II. PROPOSED HIGH-SENSITIVITY RADIATION DETECTOR

We propose to replace this conventional radiation detector with one that has far greater sensitivity for positron-emitting

isotopes but does not sacrifice the ability to separate peaks. The detector is a parallel-plane PET camera (Fig. 3) that utilizes conventional PET detector modules. Each plane has an active volume of 5 cm x 20 cm x 3 cm, the planes are separated by 3 cm, and the tubing runs in the gap between the planes. The PET camera essentially takes a movie of the peak as it moves through the tubing, each frame of the movie is shifted (in space) so the centers of the peak are aligned, then the frames are superimposed. This enables us to obtain the efficiency of a large area detector with reduced peak broadening. The length of tubing in the active detector area is considerably longer, so the sensitivity for each “peak” of activity is greatly increased. The effective velocity for the radioactivity passing through our proposed detector can be slowed down further by winding the tubing into a helix (Fig. 3 and 4b). This involves a more complex study of physical chemical properties of metabolic compounds associated with helical flow.

The detectors in this PET camera cover more solid angle and have higher gamma detection efficiency (3 cm thick of BGO compared to 1 cm thick of CsI:Tl), which further increases the sensitivity. The system also uses coincidence detection of individual gamma rays, which has improved signal-to-noise ratio and dynamic range. Based on calculations, we believe that this system will have over 40 times greater sensitivity than the conventional radiation detector.

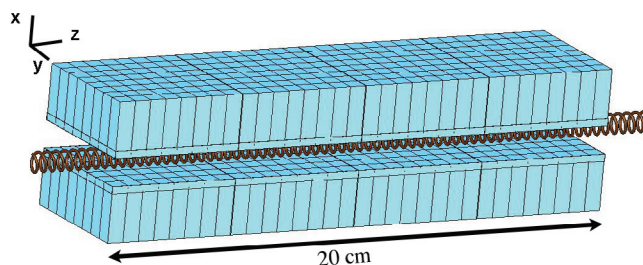


Figure 3. Proposed high-sensitivity HPLC radiation detector, showing only the active volume of the detector and tubing. The detector is comprised of parallel-plane PET modules that surround a coiled exit tube. The camera is 5x 20 cm² in area using 3 cm thick BGO scintillator. This detector has an estimated sensitivity over 40 times higher than the conventional radiation detector shown in Figure 2.

III. RESULTS

We have performed studies using a high-sensitivity HPLC radiation detector. We assembled 8 ECAT EXACT HR+ PET detector modules [3-4] into 2 parallel planes with the exit tube running along the 3 cm gap between the detector planes. The HR+ detector modules have a 1.5 cm gap between the active faces (since the HR+ module housing is wider at the base than the active front face), so the HR+ module assembly is 18.5 cm along the tube (Fig. 4b). The HR+ detector planes are readout using separate HRRT detector head interface acquisition boards, in order to acquire coincident events between all HR+ crystals in opposing detector planes [3, 5-6]. This high-sensitivity radiation detector was placed in line after the conventional radiation detector to allow a direct comparison of

measurements. The coiled exit tube that runs through the high-sensitivity radiation detector is comprised of 0.75 mm inner diameter tubing wrapped in a helical pattern around a grooved plastic rod. The tubing coils have a 14.33 mm diameter and 3.18 mm pitch, resulting in a total length of 289 cm.

The experimental setup of the HPLC system and detectors is shown in Fig. 4a. Previous HPLC studies were performed using a Phenomenex Luna 5 μ C18(2) column (250 mm x 4.60 mm), as shown within the lead shielding. However, the HPLC results presented in this paper were performed without a column to allow simple multiple injections of radiation solutions. A 20 μ l injection loop unit was used, which allowed us to inject a precisely controlled (“delta function”) volume of radioactive solution into the exit tube. A Waters model 6000A pump controlled the 70 : 30 (v/v) MeOH : H₂O solvent flow rate to 1 ml/min.

The solution was monitored sequentially by three detectors in the following order: (1) conventional ultraviolet (UV) detector, (2) conventional radiation detector, and (3) high-sensitivity radiation detector (Fig. 4a). In order to measure signal from all three detectors, the radioactivity (e.g., [¹⁸F]FDG) was diluted by the appropriate HPLC solvent mixed with an UV active factor (i.e., 11 mg/ml benzoic acid). Thus both the UV absorption and radioactive concentration were detected.

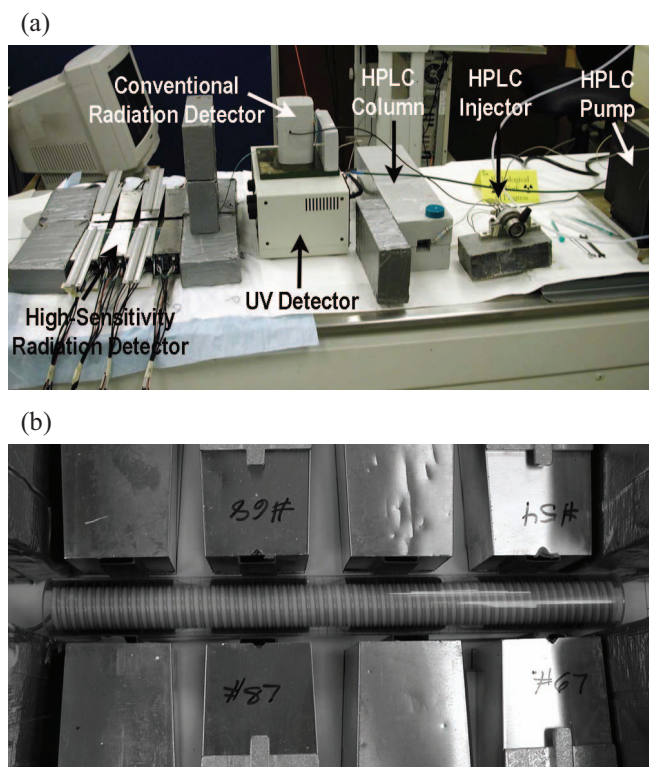


Figure 4. (a) Experimental setup used for the study. The high-sensitivity radiation detector is shown on the left. The HR+ detector modules were read out with a HRRT data acquisition system (developed for the LBNL prostate-optimized PET scanner), taking coincidence events between all crystals within opposing HR+ detector planes [6]. The HPLC system and conventional detectors are also shown. (b) Coiled exit tube that runs between two planes of HR+ detector modules.

First the solution was monitored for UV absorption using a Linear Instruments Corporation Model UV106 detector (mercury vapor lamp with 254 nm emission and effective bandwidth 1 nm; flow cell path length 6 mm and illuminated volume 9 μ l) [7]. This conventional UV detector was used to confirm that multiple injections within the same experiment had identical volumes (i.e., the area under each UV signal peak within a single data run were the same within a small error). The amplitude of the UV signal scaled with dilution concentration, and it did not reduce with time (e.g., due to radioactive decay) as did the radioactive concentration signal.

The solution was then monitored for radioactivity using both a conventional radiation detector and our high-sensitivity HR+ radiation detector (Fig. 4a). The conventional radiation detector that we used is a Carroll and Ramsey Associates Model 105S detector with a 1 cm³ CsI(Tl) crystal probe [8]. This detector was configured in the recommended sensitivity-mode configuration, with a single loop wrapped around the crystal (Fig. 2).

Figure 5 shows the signal measured by all three detectors when three 20 μ l volumes of [¹⁸F]Fluorodeoxyglucose (FDG)-benzoic acid solution were injected into the system with 1 minute time separation. Each injection had 9.3 μ Ci of radioactivity. Figure 5a demonstrates that all three injection volumes were equal, thus they should also have identical radioactive signal. For this relatively large signal, we see similarly shaped peaks with an excellent signal-to-noise ratio (SNR) from both radiation detectors, as shown in Fig. 5b and 5c. The peak width of the high-sensitivity radiation detector is larger due to dispersion in the coiled exit tube, in part since this detector measures the signal last but largely due to the coiled exit tube length. This was confirmed by placing the conventional radiation detector in line after the high-sensitivity radiation detector and measuring a commensurate increase in peak width.

Figure 6 shows the signal measured by all three detectors when three 4.1 nCi of [¹⁸F]FDG-benzoic acid solution were injected into the system with a 1 minute time separation. The signal-to-noise ratio for the high-sensitivity radiation detector signal is 27:1 for a single peak of 4.1 nCi, assuming a 110 sec peak width (Fig. 6c). For the conventional radiation detector at this low activity level, it is difficult to even determine the number of signal peaks and to estimate the variable noise. However, the SNR for the conventional radiation detector signal is about 3:1 for a single peak of 4.1 nCi, assuming a 52 sec peak width (Fig. 6b). The peak width of the high-sensitivity radiation detector is larger due to dispersion in the coiled exit tube, as discussed above.

We see an accidental coincidence noise rate of approximately 1 Hz in the high-sensitivity detector signal (Fig. 6c). We believe this is due to activation of ⁶⁸Ge in the BGO crystals from cosmic rays, since BGO crystals are contaminated by small amounts of ⁶⁸Ge [9].

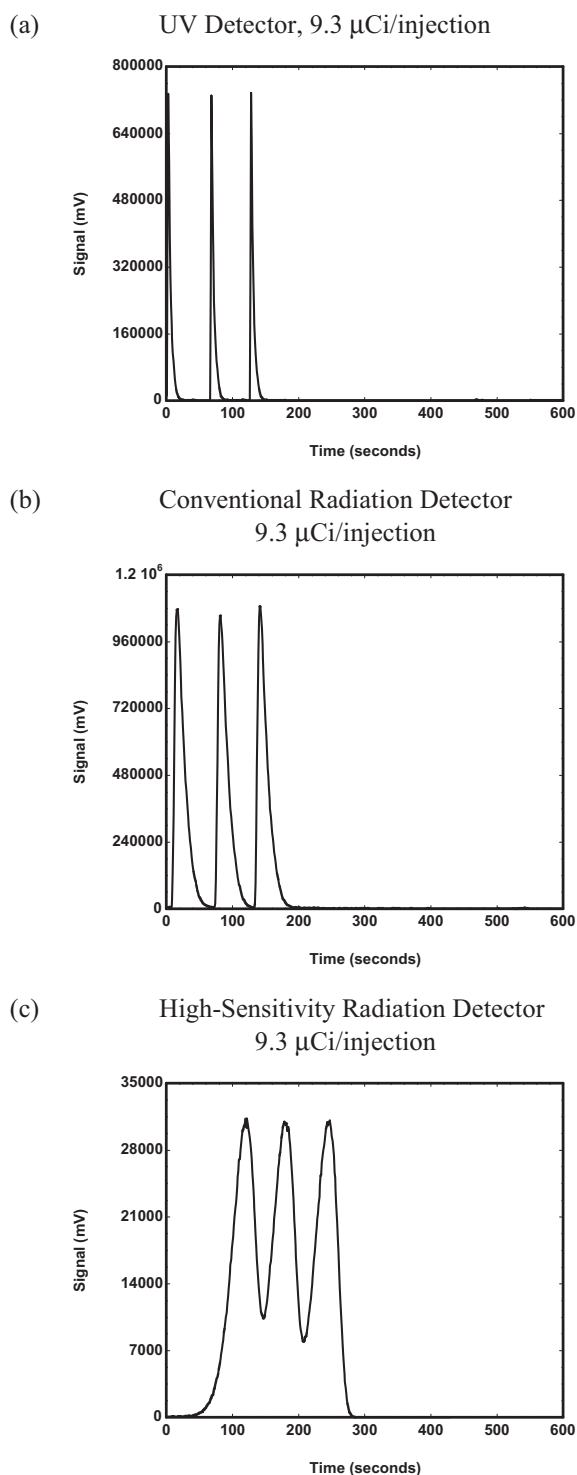


Figure 5. (a) The HPLC conventional UV detector signal when using 3 injections of 9.3 μCi [^{18}F]FDG-benzoic acid solution with a 1 minute separation. This measurement of UV absorption confirms that all three injected volumes were equal. (b) The corresponding conventional radiation detector signal. (c) The corresponding high-sensitivity radiation detector signal; the peaks are wider due to dispersion in the exit tube. All signals are plotted with 1 second time bins.

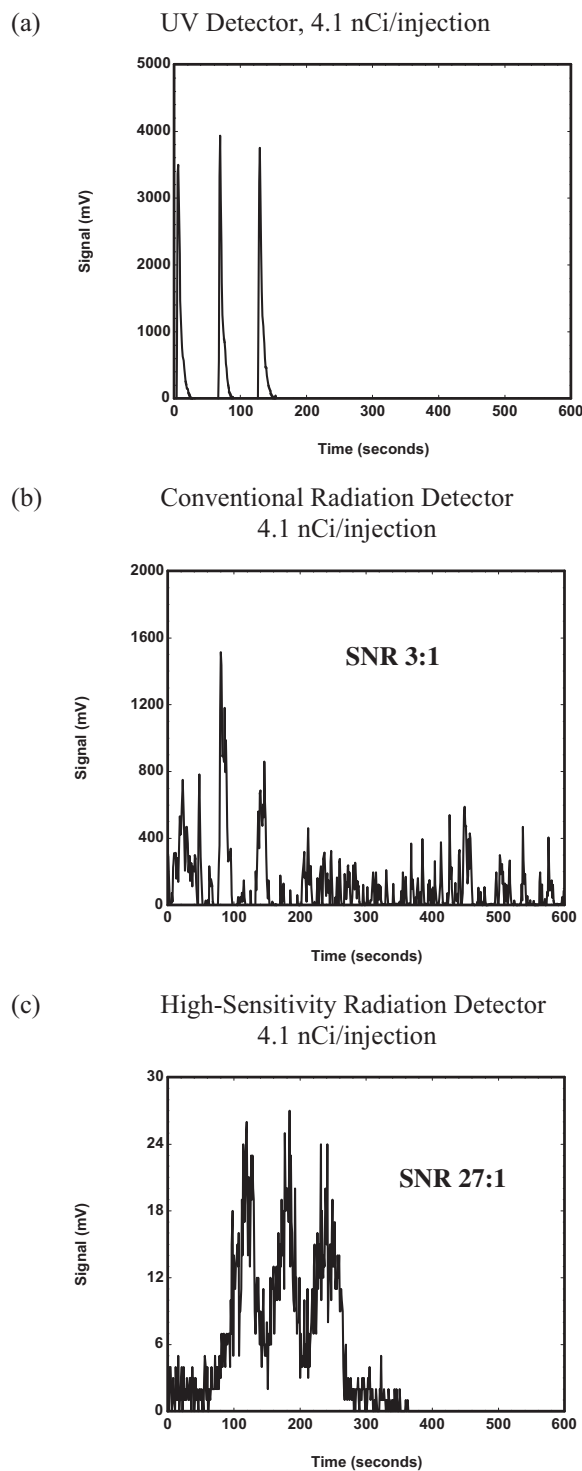


Figure 6. (a) The HPLC conventional UV detector signal when using 3 injections of 4.1 nCi [^{18}F]FDG-benzoic acid solution with a 1 minute separation. This measurement of UV absorption confirms that all three injected volumes were equal. (b) The corresponding conventional radiation detector signal. The SNR is about 3:1 for a single peak of 4.1 nCi. (c) The corresponding high-sensitivity radiation detector signal. The SNR is 27:1 for a single peak of 4.1 nCi. These peaks are wider due to dispersion in the exit tube. All signals are plotted with 1 second time bins.

IV. CONCLUSIONS AND FUTURE PLANS

We have successfully demonstrated that a high-sensitivity radiation detector, using a parallel-plane PET camera, could become an important tool for fundamental biofuel research. Using our high-sensitivity radiation detector, we have achieved a sensitivity gain of about 80 compared to the conventional radiation detector. However, the peak width of this high-sensitivity detector is larger due to dispersion in the long coiled exit tube. So we plan to model flow and dispersion for different metabolic compounds through the exit tube, in order to improve our resolution (*i.e.*, ability to separate peaks). This will be done by solving flow and diffusion partial differential equations for both linear and helical exit tube geometries. We will also investigate using different tubing diameters and solvent pump speeds.

Lastly, we will investigate imaging a HPLC column (instead of a straight or coiled exit tube) between the high-sensitivity detector planes. This should allow increased sensitivity and resolution.

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